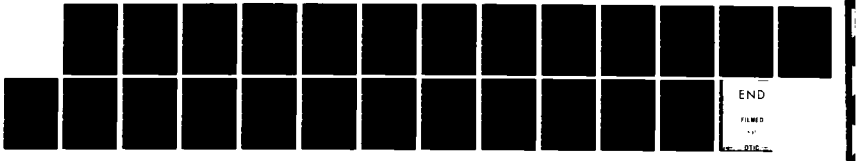
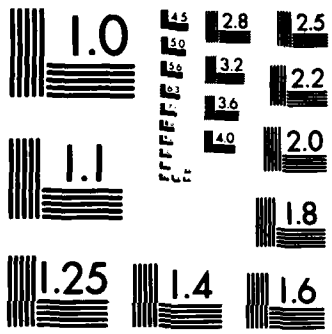
AD-A131 228 WOUND HEALING: BIOCHEMICAL PATHWAYS AND IN VIVO STUDIES 1/1
(U) WASHINGTON UNIV SEATTLE DEPT OF SURGERY
J A SCHILLING ET AL. FEB 80 DAMD17-75-C-5022

UNCLASSIFIED F/G 6/5 NL





WOUND HEALING: BIOCHEMICAL PATHWAYS AND IN VIVO STUDIES

FINAL SUMMARY REPORT

JOHN A. SCHILLING, M.D. AND PATRICK D. GOLDSWORTHY, Ph.D.

February 1980

Supported by

U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
Fort Detrick, Frederick, Maryland 21701

Contract No. DAMD 17-75-C-5022

Department of Surgery
University of Washington 98195
Seattle, Washington 98195

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

REPORT DOCUMENTATION PAGE

READ INSTRUCTIONS BEFORE COMPLETING FORM

1. REPORT NUMBER	2. GOVT ACCESSION NO. A131228	3. RECIPIENT'S CATALOG NUMBER
4. TITLE (and Subtitle) WOUND HEALING: BIOCHEMICAL PATHWAYS AND IN VIVO STUDIES		5. TYPE OF REPORT & PERIOD COVERED FINAL REPORT
		6. PERFORMING ORG. REPORT NUMBER
7. AUTHOR(s) John A. Schilling, M.D. Patrick D. Goldsworthy, Ph.D.		8. CONTRACT OR GRANT NUMBER(s) DAMD 17-75-C 5022
9. PERFORMING ORGANIZATION NAME AND ADDRESS Department of Surgery RF-25 University of Washington Health Sciences Center Seattle, Washington 98195		10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS 62772A. 3S162772A814.00.080
11. CONTROLLING OFFICE NAME AND ADDRESS US Army Medical Research and Development Command, Fort Detrick, Frederick, Maryland 21701		12. REPORT DATE FEBRUARY 1980 13. NUMBER OF PAGES 24
14. MONITORING AGENCY NAME & ADDRESS(if different from Controlling Office)		15. SECURITY CLASS. (of this report) Unclassified 15a. DECLASSIFICATION/DOWNGRADING SCHEDULE

16. DISTRIBUTION STATEMENT (of this Report)

Approved for public release; distribution unlimited

17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)

18. SUPPLEMENTARY NOTES

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)

N-acetylgalactosaminyl transferase, amino acids, carbazole, carbohydrates, chondroitin sulfate, collagen, connective tissue, Dacron Weavenit vascular graft, dog, enzymes, galactosamine, glycine, glycosaminoglycans, glycosyltransferases, hyaluronic acid, hyaluronidase, hydroxylsine, hydroxproline, ion exchange chromatography, man, oligosaccharides, proline, proteins, proteoglycans, rat, scar tissue, (continued)

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

The insoluble collagens from connective tussue experimentally induced by rat, were analyzed for amino acid composition. Essentially no differences were observed, the comparative values for these species being similar. In the rat it was found that these tissues contained three classes of heteropolysaccharides in a complex mixture of glycosaminoglycans, collagen disaccharides, and sialoglycoproteins as well as a less soluble fraction which is more intimately bound to the collagen fibers of tissue. The same three classes of carbohydrate macromolecules were found in the fascia adjacent to the experimentally induced connective tissue contained dermatin

DD FORM 1 JAN 73 1473 EDITION OF 1 NOV 65 IS OBSOLETE

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

No. 19 (continuation)

serum glycoproteins, sialoglycoproteins, structural glycoproteins, wire-mesh wound model and wound healing, ultrasound, acoustic impedance, compressional wave form, acoustic echoe, reflection coefficient, transducer, spectrum analyzer, buffer rod, acoustic lense, sound velocity.

No. 20 (continuation)

sulfates and chondroitin sulfates in addition. Dacron Weavenit cylinders were found to provide a unique wound model providing fresh tissue for immediate enzymatic studies.

New methodologies and biochemical procedures were established for the assay of N-acetylgalactosaminyl transferase (AGAT), the biosynthetic enzyme for chondroitin sulfate, which in turn exists in greater proportion than any of the other component glycosaminoglycans of wound tissue. The acceptor was prepared by digestion of chondroitin-4-sulfate with hyaluronidase and chromatographic isolation of oligosaccharide.

Stainless steel implanted-cylinder wound models and the healing of skin incision wounds were studied to determine variations in tissue concentrations of AGAT and hydroxyproline during the generation of wound tissue. AGAT concentrations in the wound model studies reached a maximum in 2 weeks in wound fluid and were higher than in wound tissue with a maximum at 4 weeks. In the wound incision studies AGAT concentrations reached a maximum in 1 week in wound tissue, were higher than in adjacent skin, and corresponded to the maximum concentrations of hydroxyproline from soluble collagen, also observed at 1 week in wound and adjacent tissues. The inflamatory effect of turpentine produced increased AGAT concentrations, over controls, in both wound and adjacent tissues.

Electrocautery wounds contained greater AGAT concentrations than scalpel incision control wounds. The lack of difference between the hydroxyproline concentrations of total collagen from scalpel wound and from non-wound tissue is thought to be due to the excision of too much non-wound tissue along with the wound connective tissue samples.

The velocity of ultrasound appeared to be the same in both wound and non-wound tissues samples. However, this apparent lack of expected velocity difference may be due to the small amount of sound transmitted through the narrow wound connective tissue being masked by the larger background of non-wound transmitted sound.

FOREWORD

We have used a simple, reproducible experimental wound model to accummulate a vast amount of information concerning wound fluid, fibroplasia and collagen-matrix formation in rat, dog, and man. The wound is induced by a stainless steel wire mesh cylinder implanted subcutaneously into the host with sterile techniques. The implanted cylinder itself evokes very little foreign-body inflammatory response. The size of the cylinder, mesh of the wire, and its gauge may be varied appropriately. In essence, the implanted cylinder creates a sterile dead space which fills with an extracellular plasma-like fluid and with fibrocollagenous tissue in an orderly and reproducible fashion. The wound induced connective tissue and component fluid may be sampled from the cylinder at any time during the time-course of fibroplasia. Tracer materials such as ^{14}C or ^{3}H labelled hexosamines, hexoses, or amino acids may be injected directly into the cylinder and followed out into the whole animal body. Conversely, radioactively tagged precursors may be injected systemically and followed within the cylinder where they are incorporated or bound. The wound fluid inside the cylinder as well as the fibrocollagenous tissue therein may be removed and analyzed qualitatively and quantitatively for chemical content, and specific components may be isolated for radioactivity measurements. The connective tissue may also be removed fresh and intact from inside the cylinder wall and assayed by explanting into tissue culture, or it may be studied by electron microscopy and electron microscope autoradiography. This fibrocollagenous tissue may be autologously, homologously, or heterologously used as a major arterial vascular substitute, as a cardiac valve, as a fascial graft, or simply placed subcutaneously to observe its growth without functional stress. Again, following explant into tissue culture or transplant, this fibrocollagenous tissue can be studied biochemically, electron-microscopically or by radioactive tracer techniques. The wound fluid and fibro-collagenous tissue inside the cylinder may be compared with the body fluids, blood serum, or body tissues outside the cylinder. The model is not complicated by infection, contracture, or epithelialization. It is analogous to a tissue culture *in vivo* of the fibroblast, a highly anabolic unit, which produces collagen and some of the glycosaminoglycans of the ground substance.

Collagen is the binding tissue of all mammals, including man, and comprises 30% of the body mass. Further, it is a key material in wound healing, without which there is no strength or permanence. It is not inert but shares in the dynamic balance of local and whole body stress, nutrition, and metabolism. There are very few proteins which contain no carbodydrate in their chemical make-up. Collagen is no exception. Collagen of connective tissue contains a small percentage of disaccharide side chains thought to serve both as intermolecular bridges and play a significant role in the maturing of the collagen fibers. The collagen fibers are imbedded in an amorphous carbohydrate matrix. This matrix has been assigned no definite structure by present electron microscope techniques, but is known to have a complex chemical nature consisting of acidic glycosaminoglycans (mucopolysaccharides) and glycoproteins (proteins with covalently bound hetero-polysaccharide chains). The matrix portion of the collagen unit is obviously important in the synthesis, maturation, and metabolism of connective tissues and the healing of wounds.

Specific Aims. During growth and development the organism continuously remodels its tissue, the "normal" process being characterized by exquisite precision as to time, place, and degree. Wound repair processes utilize these growth mechanisms. Studies of repair tissue - collagen and matrix - provide an insight into the process by which the body assembles complex reproducible structures from simple molecular units. This program has continued on the premise that studies of wound healing are very fundamental to the understanding of basic growth and are of vital importance to the preservation of life. There have been relatively few advances in the care of wounds in recent years because of this lack of understanding of basic mechanisms, stimulus, and inhibition. In an empirical, observed way, more has been learned about what not to do than what to do and why. At this juncture in time, however, a great deal is being learned about basic cellular function, growth, and regeneration and repair mechanisms on many fronts. The purpose of these studies is to contribute new knowledge, utilize improved techniques of investigation, assay appropriate new information, and apply where possible this new knowledge to the patient in the care of wounds and in the prevention of wound complications.

SIGNIFICANCE

Today, war and civilian accidental trauma place injury in the first position with regard to the causes of loss of productive life years from death and disability up to the age of 65. If one programs the above in terms of dollars, 2.5% of our Gross National Product is lost. Thus, a carefully conceived effort to prevent injury or to improve knowledge and care of wounds, whether on the battlefield, at home, or at work, may yield returns of many hundredfold in terms of manpower and dollars.

As indicated in the foregoing, our efforts are primarily directed toward a more precise and complete understanding of wound healing at the cellular level. We have made significant progress in the identification of the collagen macromolecule and its precursors, and their relationship to the dying fibroblast. The relation of the monosaccharides to the more complex mucopolysaccharides and glycoproteins and their role in the production and structure of collagen is evolving. Only through knowledge of the metabolic pathways and of key substances involved in wound repair can one direct new therapy that might accelerate wound repair and/or minimize complications.

To support the foregoing paragraphs with their clinical connotations, a major recommendation by Dr. Earl P. Benditt, Professof or Pathology, University of Washington Medical Center, Seattle, in the Trauma Workshop Report: Cell Biology of Injury and Wound Healing, printed in the JOURNAL OF TRAUMA (10:1063, 1970), is the pursuit of basic knowledge. "We recommend general expansion of basic science data. . . We know only fragments of the details of protein synthesis in the wound. . . about polymerization and aggregation of the subunits of collagen, elastin, and mucopolysaccharides, and how they relate to the gain of tensile strength."

FINDINGS

Biochemical Section

Knowledge gained over a number of years of research in animals is now being applied in man with the use of a specialized wound model to observe and analyze fibrocollagenous tissue synthesized de novo in the wound. This remarkably innocuous technique of subcutaneous implantation and excision of stainless steel wire mesh cylinders has provided wound tissue for biochemical studies and probably constitutes the first effort to structure and study this tissue in the human. The present report contains data for water, lipid, and collagen analyses of the tissue during a 16-week period of growth along with concomitant studies in the rat. The general composition of this tissue was exceedingly similar in both human and rat, but differences were noted in the greater lipid content in the human tissue and the production of more tissue per cylinder than in the rat. A depolymerization of collagen in human wound tissue developed after 12 weeks of growth while collagen in the rat remained rather stable in a similar time period.

Small amounts of carbohydrate substances are known to reside in wound tissue along with the dominant protein, collagen. These structural carbohydrates have not been characterized nor has their exact function been determined. The second biochemical study in this report has been concerned with an attempt to quantitate and clarify the functional role of carbohydrates in wound healing. Human, dog, and rat wound tissues were analyzed for hexuronic acids, sialic acids, hexosamines--galactosamine and glucosamine, and hexoses--glucose, galactose, and mannose. The monosaccharide pattern was similar in the wound tissue of the three species. These carbohydrates were found to vary during the development of the wound tissue. One of the more interesting features revealed in the study was the high hexose content and especially the uniquely high glucose content in human wound tissue. A structural carbohydrate role for glucose has been considered as unusual but has recently been reported in skin and glomerular basement membrane. This investigation has also dealt with the biochemical isolation of the structural carbohydrates. Mucopolysaccharides were extracted from wound tissue of various ages in a series of dogs. While it was originally thought that mucopolysaccharides were major structural carbohydrates in wound tissue, the current study has established that these components make up less than 25% of the total carbohydrate profile. Glycoproteins are now thought to be present in greater amounts and perhaps may be of more importance in the wound. Experiments are underway to isolate glycoproteins of wound tissue and these studies pose great value in explaining the relationships between protein and carbohydrate synthesis in wound healing.

The nature of granulation tissue differs according to the method by which it is produced. The continued success of our wound healing studies has been due to the use of a unique, yet simple, and reproducible wound model. The stainless steel wire mesh cylinder wound model simulates the uncomplicated wound and induces the synthesis of scar-type connective tissue. The model invokes the synthesis of tissue in man which appears chemically the same--especially the same carbohydrate

profile--as in the dog or rat. The total sugar content of this tissue was about 3% of the dry tissue weight in each species with the main difference being the greater amount of hexose in connective tissue of man. Sugars identified in cylinder connective tissue of man, dog or rat were glucose, galactose, mannose, fucose, glucosamine, galactosamine, sialic acid, and hexuronic acid.

Digestion of experimentally induced connective tissue of man, dog, and rat with trypsin solubilized almost 60% of the carbohydrate and peptide portions; and final digestion was accomplished with Pronase. The carbohydrate units yielded by this procedure, with only a few amino acid residues attached, were isolated by gel filtration on Sephadex G-50, Sephadex G-25, Sephadex G-15, and ion-exchange chromatography. Characterization of glycopeptides separated by gel filtration, ion-exchange chromatography, electrophoresis, paper and thin-layer chromatography indicated the presence of three distinct carbohydrate units. The first unit was the hydroxylysine-linked disaccharide of glucose and galactose which is typical of collagenous tissues but never before reported in wound tissue of any kind. The second unit was a plasma-type heteropolysaccharide consisting of galactose, mannose, glucosamine, fucose, and sialic acid. The third and largest in molecular size was the complex mixture of glycosaminoglycans composed predominantly of dermatan sulfate, chondroitin-4 and/or -6 sulfates and hyaluronic acid. The importance of these studies is quite apparent when one considers that the type and quantity of each carbohydrate unit probably plays a prominent role in both the polymerization and structural arrangement of the collagen fibers. These carbohydrates obviously influence the normal process of healing and affect the synthesis and stability of scar material.

The lysosomal enzyme neuraminidase was detected in tissue surrounding implanted cylinders as well as in tissue adjacent to the wound area in a series of rats. The cylinders were implanted in rats for time periods of 1, 2, 3, and 4 weeks. The highest level of neuraminidase was found in outer tissue of cylinders implanted for a one-week period and the activity decreased thereafter. These results imply that lysosomal hydrolases, e.g., neuraminidase, are released and are active in the wound area and therefore play a role in inflammation and perhaps influence wound healing. Additional work will be required to substantiate this data with more experiments in an attempt to locate the cell type and particulate location of the enzyme. Studies involving Dacron prostheses are being employed to aid in accumulation and ease of removal of tissue within the wound for assays of this enzyme. The analyses of such an enzyme in and around the wound provides an important new dimension for studies of wound healing. The time of synthesis or activation of these enzymes correlated with the time-course of healing would be crucial for assaying proper development and organization of the involved tissues.

Electron Microscope Studies

A morphologic and dimensional analysis of the molecular structural subunit of the native collagenic fibril has been completed and is included "in toto" in this report under the title "The collagen molecule: A new concept of its configuration as the structural subunit of the native collagen fibril." Quantitative

studies have been made on E.M. demonstrable corpuscular structural subunits of collagen fibrils of the long dermis, Achilles tendon, and induced fibrocollagenous tissue complexes. The subunit is shown to consist of a three-stranded cable structure which is 22.9 A. in diameter by 3316 - 2580 A. long. The latter is macrohelically coiled asymmetrically to form a gross pear-shaped corpuscular subunit. The corpuscular subunit measures 57.2 A. and 112.3 A. in its narrowest and widest diameters respectively, and 263.2 - 269.0 A. in length. Each secondary strand of the cable has a mean diameter of 10.2 A. and calculated length of 3316 - 2580 A. The dimensionally characterized components and form features of the corpuscular subunit are directly and closely correlable with all wide- and low-angle meridional and equatorial x-ray diffraction spacings so far reported on vertebrate collagen. Calculated molecular weight values for the dimensionally characterized form features, i.e., the cable, secondary strands, and for the dimensionally characterized form features, i.e., the bulbous portion, the thin portion, and macrohelical cable turns, are all directly and closely correlable with reported biochemical and physical chemical data on the length, diameter and molecular weight of the solubilized collagen molecule, its individual alpha-chains, and the major and minor sub-fragments thereof. The directness and closeness of the correlations indicate that the asymmetric corpuscular subunit characterized in the native solid state collagen complex is to be identified with the collagen molecule in solution.

Certain facets of work on reconstituted collagen have been completed under the title of "Ultrastructural studies on the genesis of reconstituted collagen." A fine globular structure characterizes all stages in the genesis of SLS (segment-long-spacing) from the least to the most highly differentiated stages. The definite cross-banding pattern of reconstituting SLS forms is time-dependent. Cross-bands appear first only at the ends of the SLS segments; the centers become progressively more striated over time. The lengths of reconstituting SLS vary with time and are also variable both within the limits of one SLS segment and from one segment to another (e.g., 1824 A^{o} to 2292 A^{o}). The widths of reconstituting SLS vary with time; they also vary within the limits of one SLS and from one segment to another. The cross-banding pattern is subject to mechanical deformations that are inconsistent with currently accepted theories of collagen ultrastructure. The morphologic and quantitative data presented on the emerging segment-long-spacing type of collagen is not consistent with the hypothesis that fibrillogenesis involves a side-by-side alignment of structural subunits possessing the dimensions of a long rod-shaped macromolecule. Rather, the structural subunit involved is of a globular form.

Specific aspects of our work on the induced human fibrocollagenous tissue complexes have been completed. An illustrated summary of part of this work is included in this report under the title: "General and ultrastructuring of induced human fibrocollagenous tissue complexes." Morphologic studies of human connective tissue complexes utilizing both light and electron microscopy methods have yielded a complex consisting of alternating cell dominated and fiber dominated zones. The dominant cell type of the fibrous zones descriptively resembles cells undergoing wasting or cell death. A honocrine-like deterioration in collagenic fibril formation is manifest in these cells. The dominant cell type of the cellular zones exhibit an apocrine-like functional appearance in collagenic fibril formation. Descriptively, the human connective tissue complex is basically the same as that of the complex induced in the dog with some minor changes.

Studies on thermal effects on Tropo-Collagen molecular configuration and integrity have utilized acid-soluble TC obtained from carp swimbladder. TC solutions treated for 2 hours at temperatures ranging up to ambient temperatures of 25-30° C reconstitute into usual SLS fibres while maintained at these temperatures, and establish that in this range any thermal transformation does not alter the basic molecular integrity of the TC. TC solutions maintained at 37.° C for 2 hours show a transformation of rod-form TC (at 5.0°C) into asymmetric pyriform units. Measurements on these particles (determined by particle-height:shadow-length relationships, and particle dimension at right-angles to the direction of shadowing) show them to have diameters between 50 and 150 A and lengths of 200-290A. Formation of SLS fibres in systems maintained at 37.0°C appears not to occur. However, when solutions treated for 2 hours at 37.0°C are treated with ATP and re-cooled to 5.0°C, SLS fibres are reconstituted. The asymmetric transformed TC observed in our thermal studies have dimensions corresponding closely to those of pyriform units characterized in developing and mature native mammalian fibres. Clearly, these thermally transformed TC molecules do not represent TC-molecules in a random coiling configuration as suggested by the Engel and Grassman studies previously noted. Inasmuch as the SLS fibre appears to be derived by the orderly assembly of pyriform units, it would further appear that the transformation of rod-form TC into coiled pyriform TC may be evoked by other agents (ATP, chondroitin-sulfate, pH) in the initial stages of fibre reconstitution. This currently is under investigation.

Studies on the structuring and growth aspects of natural collagen fibres and reconstituting SLS fibres show that although there is a great species gap, there is a close similarity in the morphologic aspects, dimensional and growth increments characterizing the emergence of native dog collagen fibres in vivo and of carp SLS fibres in vitro. Further, these data similarities appear clearly not referable to technical manipulations as they are based on biological material prepared for study and analysis by totally unlike technical methods of preparation and visualization. It seems permissible to draw from these studies: 1) that the basic structuring units of both native dog fibres and carp SLS fibres are pyriform or corpuscular TC molecules; 2) that the lateral packing of these units is basically along an hexagonal plan; 3) from geometric analyses made so far on the initial stages in fibre emergence it appears that the width increment of about 90A is compatible only with the packing of particles having the specific gross structural and dimensional features of pyriform or corpuscular TC molecules. The geometry of the linear packing of pyriform units into fibres is incomplete.

The results reported here from initial studies on the effect of NaOH on reconstituted SLS fibres clearly parallel and confirm those obtained on natural collagen fibres by electron microscopic, x-ray diffraction and biochemical approaches. Further, they demonstrate that the periodic band pattern of reconstituted and fully differentiated SLS fibres are dependent on the specific packing of corpuscular (or pyriform) TC units into linear fibrillar assemblies and on the specific steric orientation of the latter in the normal mature SLS fibre. The sequence shown in this report on the total transformation of SLS fibres into a dispersed system of fibrilli having diametral and length features comparable to those of acid solubilized rod-form TC under low thermal conditions is convincing evidence that NaOH under the conditions defined here: 1) destroys the normal

bonding and packing arrangement of TC molecules in the SLS fibre and its sub-fibrillar assemblies, and 2) effects at higher concentrations (0.05N - 0.1N) the eventual transformation of pyriform TC molecules into essentially rod-form TC molecules.

The findings on the compatibility of TC molecules of differing species-of-origin in the fabrication of collagen fibres in vitro are completely consistent with previous data and analyses indicating that the basic structural unit of SLS and native fibres is a TC-molecule of pyriform configuration, and that the distinctive bands of these fibres reflect specific dimensionally characterized morphologic features of these units and their group packing arrangement in the ordered fibre. The occurrence of both X---Y=X---Y (asymmetric) and X---Y=Y---X=X---Y junctioned SLS fibres in the normal (un-irradiated) systems is specifically consistent with our data on the genesis of native and SLS fibres that laterally contiguous pyriform TC are assembled in an anti-polar orientation. They also indicate that the band pattern per se may not be used as an index of a rod-form configuration of the TC molecule or as an index of the lateral and linear packing and orientation arrangement of the TC-molecules in the ordered fibre as postulated by Hodge and Schmitt.

New observations on the genesis of SLS and native collagen fibres in this report describe findings on: (I) time-sequence studies of carp SLS genesis in standard reconstitution systems showing that the size, shape and band features of mature SLS fibres are time dependent and acquired during SLS emergence. Data on induced dog fibrocollagenous systems (II) show native fibres to arise by an ordering of units 50-108A X 263-270A. Their aggregation yields transitory fibres having irregular stepped long profiles and x-sectional profiles which range from polygonal and hexagonal and finally to cylindric configurations. Measurements on transitory through mature fibres indicate that native fibre diameter increases at an increment of 84-92A. Overall, these findings indicate a need to re-appraise current popular beliefs on the genesis and structuring of SLS and native collagen fibres, and on the significance of SLS as a "molecular fingerprint" of solubilized TC molecules.

The growth, development, and cellular activity of specially induced fibrocollagenous tissue complexes in humans has been studied during the time-course of fibroplasia utilizing both light and electron microscopic procedures. The developed fibrocollagenous tissue complexes reflect the essential size and shape of the induction device. Tissue complexes retrieved after 2 to 3 weeks of development consist primarily of fibroblasts. The tissue complexes reclaimed after 4 to 16 weeks of development demonstrate ordered lamellations consisting of zones composed predominantly of fibroblasts and zones consisting primarily of collagenous fibers. The development of the lamellations is referable to the specific fibrillogenic activities by the constitutent fibroblasts. The sequence of the morphological aspects of fibrillo-genesis occurring in the cellular zones is indicative of an initial apocrine-like role by the fibroblast. Continued fibrillogenesis by the fibroblast eventually terminates in a holocrine-like role resulting in cytodestruction and concurrent formation of a predominantly collagenic fibrous zone. Fibroblasts active in fibrillogenesis exhibit sequential changes in

in the cytoplasm involving hypertrophy of the hyaloplasm either locally in the apocrine-like role or generally in the holocrine-like role. The hypertrophy is presumably due to the accumulation of precursory collagenic material within the hyaloplasm. Subsequently, the plasma membrane disappears either locally or generally and overt collagenic fibrils form in the hypertrophied hyaloplasm. The plasma membrane reforms internal to the hypertrophied hyaloplasmic mass in the apocrine-like function depositing the organizing collagenic fibrils and the hyaloplasmic mass extracellularly. The plasma membrane is not reformed in the holocrine-like function. The deposition of hyaloplasm and organizing fibrils is continuous as the cell terminates itself by cytodestruction. The organizing collagenic fibrils continue to grow and differentiate intercellularly presumably by the incorporation of precursory collagenic material in the inter-fibrillar hyaloplasm. The organizing collagenic fibrils are related to argyrophilic fibrils supporting the concept that certain reticular fibers are collagenic in nature. The morphological aspects of fibrillogenesis supports the concept that the fibroblast functions in the synthesis and organization of collagenic fibrils. Further, both the intra- and intercellular concepts of fibrillogenesis is suggested. The structural relationship of the hypertrophic fibroblast cytoplasm and the emerging collagen fibrils supports and extends the broad concept that fibroblast contact is essential to fibrillogenesis.

New methodologies and biochemical procedures were established for the assay of N-acetylgalactosaminyl transferase (AGAT), the biosynthetic enzyme for chondroitin sulfate, which in turn exists in greater proportion than any of the other component glycosaminoglycans of wound tissue. The acceptor was prepared by digestion of chondroitin-4-sulfate with hyaluronidase and chromatographic isolation of oligosaccharide.

Stainless steel implanted-cylinder wound models and the healing of skin incision wounds were studied to determine variations in tissue concentrations of AGAT and hydroxyproline during the generation of wound tissue. AGAT concentrations in the wound model studies reached a maximum in 2 weeks in wound fluid and were higher than in wound tissue with a maximum at 4 weeks. In the wound incision studies AGAT concentrations reached a maximum in 1 week in wound tissue, were higher than in adjacent skin, and corresponded to the maximum concentrations of hydroxyproline from soluble collagen, also observed at 1 week in wound and adjacent tissues. The inflammatory effect of turpentine produced increased AGAT concentrations, over controls, in both wound and adjacent tissues.

Electrocautery wounds contained greater AGAT concentrations than scalpel incision control wounds. The lack of difference between the hydroxyproline concentrations of total collagen from scalpel wound and from non-wound tissue is thought to be due to the excision of too much non-wound tissue along with the wound connective tissue samples.

The velocity of ultra-sound appeared to be the same in both wound and non-wound tissues samples. However, this apparent lack of expected velocity difference may be due to the small amount of sound transmitted through the narrow wound connective tissue being masked by the larger background of non-wound transmitted sound. AGAT concentrations reflect the degree of inflammation in a manner

analogous to the relation of hydrosyproline to fibroplasia and collagen production. It is, however, too cumbersome for commensuration of wound healing, and not precise enough. Ultra-sound holds real promise clinically as it is non-invasive and readily applicable and can show defects in healing wounds.

PUBLICATIONS

Shetlar, M.R., Lacefield, E.G., White, Betty N., and Schilling, John A. "Wound Healing: Glycoproteins of Wound Tissue. I. Studies of Hexosamine, Hexose, and Uronic Acids Content." Proc. Soc. Exp. Biol. Med., 100:501-503, March 1959.

White, B.N., Shetlar, M.R., Shurley, H.M. and Schilling, J.A. "Wound Healing: Investigation of Proteins, Glycoproteins, and Lipids of Experimental Wound Fluid in the Dog." Proc. Soc. Exp. Biol. Med. 101:353-356, June 1959.

Schilling, John A., Joel, Walter, Shurley, Helen M. "Wound Healing: A Comparative Study of the Histochemical Changes in Granulation Tissue Contained in Stainless Steel Wire Mesh and Polyvinyl Sponge Cylinders." Surgery. 46:702-710. October 1959.

Schilling, John A. Chapter 3. In Complications in Surgery and Their Management. Edited by: Artz, Curtis P., and Hardy, James D. W.B. Saunders Co. June 1960.

Schilling, John A., Shurley, H.M., Joel, W., Richter, K.M., and White, B.N. "Fibrocollagenous Tubes Structured in Vivo. Morphology and Biological Characteristics." A.M.A. Arch. Path. 71:548-553. May 1961.

White, Betty N., Shetlar, M.R., Schilling, John A. "The Glycoproteins and Their Relationship to the Healing of Wounds." Ann. N.Y. Acad. Sci. 94:297-307. August 1961.

Shetlar, M.R., Bradford, Reagan, Hern, Danny, Endecott, Boyd, and Schilling, John A. "Fate of Radioactive Glucosamine Administered Parenterally to the Rat." Proc. Soc. Exp. Biol. Med. 109:335-337, February 1962.

Rosen, Hyman, Levenson, Stanley M., Sabatine, Paul L. Horowitz, Richard E., Shurley, Helen M., and Schilling, John A. "In Vitro Acceleration of Cell Division by Ultrafiltrate of Canine Wound Fluid." J. Surg. Res. 2:146-150. March 1962.

Richter, K.M., Schilling, J.A., and Shurley, H.M. "Internal Organization of the Collagenic Fiber." J. Appl. Phys. 35:3900. 1964.

Richter, K.M., Schilling, J.A., and Shurley, H.M. "Ultrastructure of Experimentally Induced Scar Tissue." J. Appl. Phys. 35:3099. 1964b.

Richter, K.M., Schilling, J.A., and Shurley, H.M. "Assay of Ultrafiltrates of the Dog Pre-wound-serum, -plasma, and 7-day-wound-fluid, -serum and -plasma on L-strain fibroblast and proliferation. Proceedings 15th Annual Meeting Tissue Cult. Assoc. p. 51. 1964c.

Schilling, John A., Shurley, Helen M., Joel, Walter, White, Betty N., Bradford, Reagan H. "Abdominal Aortic Grafts: Use of in vivo Structured Autologous and Homologous Fibrocollagenous Tubes." Ann. Surg. 159:819-828, June 1964.

Richter, K.M., Schilling, J.A., Shurley, H.M. "Electron Microscopic Studies on the Morphologic Relations of Fibrocytes and Collagen Fibers in Developing Experimental Scar Structures." Amer. Zoologist. 4:343, 1964.

White, Betty N., Shetlar, M.R., Shurley, H.M., and Schilling, J.A. "Incorporation of [1-^{14}C] Glucosamine into Mucopolysaccharides of Rat Connective Tissue." Biochim. Biophys. Acta. 101:97-105, 1965.

White, Betty N., Shetlar, M.R., Shurley, H.M., and Schilling, J.A. "Incorporation of D-[1-^{14}C] Galactosamine into Serum Proteins and Tissues of the Rat." Biochim. Biophys. Acta. 101:259-266, 1965.

Schilling, John A. "Technique of Implanted Steel Mesh Cylinders in Studies of Fibroplasia." Wound Healing, Proceedings of a Workshop. National Academy of Sciences, National Research Council, Washington, D.C., p. 270-272. 1966.

Richter, K.M., Schilling, J.A., and Shurley, H.M. "A Possible E.M. Demonstration of Part of the Intramacromolecular Character of the Tropocollagen Molecule." Electron Microscopy. 2:131, 1966.

Schilling, John A. "Wound Healing." Physiol. Rev. 48:374-423, April 1968.

Richter, Kenneth M., and Schilling, John A. "The Fine Structure of an Experimentally Induced Fibrocollagenous Tissue Complex." Ann. Surg. 169:10-34, January 1969.

_____, "The Fine Structure of an Experimentally Induced Fibrocollagenous Tissue Complex." Illustrated Abstract. In: Yearbook of General Surgery. Edited by: M.E. Debakey. P. 122-126, 1969.

Schilling, John A., White, Betty N., Lockhart, M.S., Shurley, H.M. "Wound Healing in the Dog. Radioisotope Studies of Developing Connective Tissue and Fluid in an Artificial Dead Space." Amer. J. Surg. 117:330-337, March 1969.

Richter, K.M., and Schilling, J.A. "A Dimensionally Characterized Corpuscular Subunit of Solid State Collagen and Its Equivalence to the Collagen Molecule of Solution." Microscopie Electronique. 1:637-638, 1970.

Shetlar, M.R., Shurley, H., Hern, D., Ellis, N., and Linares, H. "A New Model System for the Study of Wound Healing." Proc. Soc. Exp. Biol. Med. 136:664-667. 1970.

Lichti, E.L., Schilling, J.A., and Shurley, H.M. "Wound Fluid and Plasma Zinc Levels in Rats During Tissue Repair." Amer. J. Surg. 123:253-256. March 1972.

White, B.N., Lockhart, M.S., and Schilling, J.A. "Comparative Studies of Glycosaminoglycans and Glycoproteins of Experimentally Induced Connective Tissue in the Human, Dog, and Rat." Federation Proc. 31(2):648. 1972.

White, B.N., Lockhart, M.S., and Schilling, J.A. "Identification of Specific Glycopeptides and Glycosaminoglycans in Experimentally Induced Connective Tissue of Man, Dog, and Rat." Federation Proc. 32(3):830. 1973.

White, Betty N., Lockhart, M.S., and Schilling, John A. "A Quantitative Comparison of Carbohydrates in Experimentally Induced Connective Tissue of Man, Dog and Rat." Comp. Biochem. Physiol. 48B:315-320. 1974.

Stinson, W.W., Richter, K.M., and Schilling, J.A. "The Fine Structure of Experimentally Induced Connective Tissue Complexes in the Human." Ann. Surg. 180:51-66, July 1974.

Schilling, John A. "Wound Healing and Inflammatory Response." In: Surgery in the Aged. Edited by: L.J. Greenfield. Philadelphia: W.B. Saunders Co. p. 24-38. 1975.

White, B.N., Lockhart, M.S., and Schilling, J.A. "Nature of the Carbohydrate Units of Experimentally Induced Connective Tissue of Man, Dog, and Rat." Comp. Biochem. Physiol. 50B:411-418. 1975.

Schilling, John A. "Wound Healing." Surg. Clin. N. Amer. 54:859-874, August 1976.

Schilling, John A., and Heimbach, David M. "Wound Complications." Am. Surg. 43:682-685. 1977.

John A. Schilling, M.D.

WOUND HEALING

Annual Reports

1. Fractional Analysis of Experimental Wound Fluid: Chemical Studies of Wound Fluid and Serum and Regenerating Tissue, and Infrared Studies.
   John A. Schilling, Betty N. White, and Marvin R. Shetlar.
   Annual Report, Dept. of the Army, Contract No. DA-49-007-MD-747, 15 July 1957.

2. Fractional Analysis of Experimental Wound Fluid: Investigation of the Proteins, Glycoproteins, and Lipids of Experimental Wound Fluid and Connective Tissue.
   John A. Schilling, Marvin R. Shetlar, Walter Joel, Betty N. White, and Helen M. Shurley.
   Annual Report, Dept. of the Army, Contract No. DA-49-007-MD-747, 15 July 1958.

3. Fractional Analysis of Experimental Wound Fluid.
   John A. Schilling, Betty N. White, Helen M. Shurley, Marvin R. Shetlar, and Walter Joel.
   Annual Report, Dept. of the Army, Contract No. DA-49-007-MD-747, 15 July 1959.

4. Fractional Analysis of Experimental Wound Fluid: Further Studies of the Analysis and Characterization of Wound Fluid, Histochemical Studies of Wound Tissue, and Chemical Investigation of Mucopolysaccharides of Wound Tissue and Wound Fluid.
   John A. Schilling, Betty N. White, Helen M. Shurley, Anna Price, Marvin R. Shetlar, and Walter Joel.
   Annual Report, Dept. of the Army, Contract No. DA-49-007-MD-747, 15 July 1960.

5. Fractional Analysis of Experimental Wound Fluid.
   (a) Glycoproteins and Their Relationship to Healing of Wounds.
   (b) Additional Experiments Involving Comparative Use of Various Materials for Cylinder Implantation.
   (c) Continued Studies of Fibrocollagenous Tubes Structured in Vivo.
   (d) Studies of Effect of Altered Oxygen Tension on Healing of Wounds.
   (e) Growth Stimulation by the Ultrafiltrate of Wound Fluid.
   (f) Tracer Studies Utilizing Glucosamine-1-C^{14} in Rats.
   John A. Schilling, Betty N. White, Helen M. Shurley, Anna Price, Marvin R. Shetlar, and Walter Joel.
   Annual Report, Dept. of the Army, Contract No. DA-49-007-MD-747, 15 July 1961.

John A. Schilling, M.D.

WOUND HEALING

Annual Reports

6. Fractional Analysis of Experimental Wound Fluid
   (a) In vivo Studies of Glucosamine-1-C-14 in Rats
   (b) Studies of N-Acetylglucosamine-1-C-14 in Rats
   (c) Studies with D-Galactosamine-1-C-14 in the Rat
   (d) Autologous and Homologous Fibrocollagenous Aortic Grafts
   (e) Aortic Value Substitutes
   (f) Tracheal Substitutes
   John A. Schilling, Betty N. White, Marvin R. Shetlar, Walter Joel, Frances G. Felton
   Annual Report, Dept. of the Army, Contract No. DA-49-007-MD-747, 15 July 1962.

7. Fractional Analysis of Experimental Wound Fluid
   (a) Incorporation of Glucosamine-1-C-14 by Rat Connective Tissue in vivo
   (b) Arterial Grafts and Sulfur-35 Studies
   (c) In Vitro Assays Using Lactobacillus Casei
   (d) In Vitro Assays Using Strain-L Mouse Fibroblasts
   John A. Schilling, Betty N. White, Helen M. Shurley, Walter Joel, Frances G. Felton, Marvin R. Shetlar, Kenneth M. Richter
   Annual Report, Dept. of the Army, Contract No. DA-49-007-MD-747, 15 July 1963.

8. Fractional Analysis of Experimental Wound Fluid
   (a) Biochemical Investigation of the Healing Wound Using Radioactive Tracer Substances
   (b) In Vivo Synthesis and Structuring of Fibrocollagenous Tissue for Surgical Repair and Transplantation
   (c) Continued Studies of Fractionated Wound Fluid, Including Characterization of the Growth Regulating Factors
      1. Further Studies with an Unidentified Growth Factor for Lactobacillus Casei in Experimental Wound Fluid Fractions
      2. Electron Microscopic Assay of Fibroblast Collagen and Mucopolysaccharide Synthesis

   John A. Schilling, Betty N. White, Helen M. Shurley, Walter Joel, Kenneth M. Richter
   Annual Report, Dept. of the Army, Contract No. DA-49-007-MD-747, 15 July 1964.

9. Fractional Analysis of Experimental Wound Fluid
   (a) Biochemical Investigation of the Healing Wound
      1. Studies of Mucopolysaccharides and Glycoproteins
      2. Interrelationship of Collagen and Ground Substance Synthesis during Healing

John A. Schilling, M.D.

WOUND HEALING

Annual Reports

(b) In Vivo Synthesis and Structuring of Fibrocollagenous Tissue for Surgical Repair, Transplantation, and Electron Microscopic Studies.
1. Studies Relating to Aging of Fibrocollagenous Tissue
2. Heart Valve Repair and Replacements
3. pH of Interior of Subcutaneously Implanted Stainless Steel Wire Mesh Cylinders

(c) Studies of Wound Fluid Ultrafiltrate
1. Stimulation of Growth of Lactobacillus Casei by Wound Fluid Ultrafiltrate
2. In Vitro Assay Using Strain-L Fibroblasts

(d) Electron Microscopic Analyses Related to Collagen and Mucopolysaccharides of Fibrocollagenous Tissue

John A. Schilling, Betty N. White, Kenneth M. Richter, Walter Joel, John R. Sokatch, Helen M. Shurley, Marion Lockhart.

Annual Report, Dept. of the Army, Contract No. DA-49-007-MD-747, 31 December 1965.

9a. Fractional Analysis of Experimental Wound Fluid.

A Ten-Year Progress Report 1956-1966.

February, 1966.

10. Wound Healing: Biochemical Pathways, Ultrastructure, and Clinical Studies

(a) In Vivo Synthesis of Fibrocollagenous Tissue and Production of Wound Fluid
1) Studies in the Human
2) Studies of Wound Fluid Relating to Zinc
3) In Vitro Assay Using Strain-L Fibroblasts
4) Physical Constants of Internal Environment

(b) Biochemical Investigation of the Healing Wound
1) The Role of Structural Carbohydrates in Wound Healing
   a. Incorporation of [1-14C]Glucosamine by Rat Connective Tissue: Nature of the Process in Vitro
   b. Incorporation of [1-14C]Glucosamine by Rat Connective Tissue: Changes Related to Developmental Age of Tissue
   c. Comparison of the Utilization of [1-14C]Glucosamine and [1-14C]Galactosamine by Rat Connective Tissue
2) The Carbohydrate Composition of Developing Connective Tissue
   a. Isolation and Purification of Specific Mucopolysaccharides of Dog Connective Tissue

(c) Electron Microscope Studies

John A. Schilling, Betty N. White, M.S. Lockhart, Helen M. Shurley, Edgar M. Lichti, Carl A. Nau, Kenneth M. Richter, Charles Palmer, W. Joel

Annual Report, Dept. of the Army, Contract No. DA-49-007-MD-747, 31 December 1966.

John A. Schilling, M.D.

WOUND HEALING

Annual Reports

11. Wound Healing: Biochemical Pathways, Ultrastructure, and Clinical Studies
    (a) In Vivo Synthesis of Fibrocollagenous Tissue and Production of Wound Fluid
        1) Studies in the Human
        2) Studies of Wound Fluid Relating to Zinc
            a. Zinc Transport to Surgical Wound Sites
            b. A Profile of Plasma and Wound Fluid Zinc Levels After Implantation of Non-Metallic Cylinders in Rats and a Healthy Mongrel Dog
    (b) Biochemical Investigation of the Healing Wound
        1) A Comparative Study of Induced Wound Connective Tissue in the Human and Rat: Changes in Water, Lipid, and Dry Weight Composition in Relation to Growth-Age of the Tissue.
        2) The Role of Carbohydrates in Wound Healing: Studies of Hexosamine Metabolism by Wound Components of the Rat Using [1-14C]Glucosamine and [1-14C]Galactosamine.
        3) Studies of Developing Connective Tissue and Fluid of a Wound Using [1-14C]Glycine, and [U-14C]Proline.
    (c) Electron Microscope Studies
        1) Current Studies
        2) The Fine Structure of an Experimentally Induced Fibrocollagenous Tissue Complex

    John A. Schilling, Betty N. White, M.S. Lockhart, H.M. Shurley, E.L. Lichti, C.M. Nau, K.M. Richter, Charles Palmer, Walter Joel.
    Annual Report, Dept. of the Army, Contract No. DA-49-193-MD-2811, 15 April 1968.

12. Wound Healing: Biochemical Pathways, Ultrastructure, and Clinical Studies
    (a) Biochemical Investigation of the Healing Wound
        1) Wound Granulation Tissue in the Human: Compositional Changes in Relation to Tissue Age
        2) The Carbohydrate Composition of Developing Connective Tissue: A Comparative Carbohydrate Profile of Induced Wound Connective Tissue of Varying Age in the Human, Dog, and Rat
    (b) Electron Microscope Studies
        1) Quantitative E.M. Data on a Collagenic Fibril Structural Subunit, the Collagen Macromolecule
        2) The Light Microscopic Aspects of the Induced Human Scar Fibrocollagenous Tissue Complex

    John A. Schilling, B.N. White, Micki Lockhart, Judy Antonetti, K.M. Richter, Charles Palmer, Walter Joel, C.A. Nau.
    Annual Report, Dept. of the Army, Contract No. DA-49-193-MD-2811, 15 April 1969.

John A. Schilling, M. D.

WOUND HEALING

Annual Reports

13. Wound Healing: Biochemical Pathways, Ultrastructure, and Clinical Studies
    (a) Biochemical Investigation of the Healing Wound
        1. Wound Granulation Tissue in the Human: Protein, Lipid, Dry Matter and Water Changes in Relation to Tissue Age
        2. Wound Granulation Tissue in the Human: Carbohydrate Composition in Comparison to that of the Dog and Rat
    (b) Electron Microscope Studies of the Healing Wound
        1. The Collagen Molecule: A New Concept of Its Configuration as the Structural Sub-unit of the Native Collagenic Fibril
        2. Ultrastructural Studies on the Genesis of Reconstituted Collagen
        3. General and Ultrastructuring of Induced Human Fibrocollagenous Tissue.

    John A. Schilling, B.N. White, Mickie Lockhart, K.M. Richter, Charles Palmer, Walter Joel, Carl A. Nau.

    Annual Report, Dept. of the Army, Contract No. DA-49-193-MD-2811, 15 April 1970.

14. Wound Healing: Biochemical Pathways, Ultrastructure, and Clinical Studies
    (a) Biochemical Investigation of the Healing Wound
        1. Carbohydrate Composition of Wound Granulation Tissue and Wound Fluid in the Human: Comparison to that of the Dog and Rat
    (b) Electron Microscope Studies of the Healing Wound
        1. A Corpuscular Structural Unit of Solid State Collagen and Its Equivalence to the Collagen Molecule of Solution
        2. Studies of the Growth, Development, and Cellular Activity of Experimentally Induced Connective Tissue Complexes in the Human
        3. Ultrastructural Studies on Solubilized and Reconstituted Collagens

    John A. Schilling, B.N. White, M.S. Lockhart, K.M. Richter, Charles Palmer, C.A. Nau, W.W. Stinson, Ralph Day

    Annual Report, Dept. of the Army, Contract No. DA-49-193-MD-2811, 15 April 1971.

15. Wound Healing: Biochemical Pathways, Ultrastructure, and Clinical Studies
    (a) Biochemical Investigation of the Healing Wound
        1. Functional Aspects of the Carbohydrates of Wound Healing
        2. Discovery, in the Course of Routine Analytical Procedures, of Unique Dicarbonyl Carbohydrates Useful as Glucose Antimetabolites
    (b) Electron Microscope Studies of the Healing Wound
        1. An Analysis of the Packing Arrangement of Collagen Molecules in Native and Reconstituted Collagen Fibrils

John A. Schilling, M.D.

WOUND HEALING

Annual Reports

2. EM Studies on the Thermal Transformation of Collagen Molecules and Their Capacity to Reconstitute Solid State Fibrils
3. Experimental EM Studies on the Effect of NaOH on the Morphology of the Collagen Molecule or Structural Unit of Solid State Complexes
4. Analytic Studies on the Structuring of Atypical Collagenic Tissue Structures
5. An EM Analysis of the Influence of Species Differences in the TC-molecule on the Structuring of Reconstituted Fibrils

John A. Schilling, B.N. White, M.S. Lockhart, K.M. Richter, Charles Palmer, C.A. Nau.

Annual Report, Dept. of the Army, Contract No. DA-49-193-MD-2811, 15 June 1972.

16. Wound Healing: Biochemical Pathways, Ultrastructure, and Clinical Studies

(a) Biochemical Investigation of the Healing Wound

1. A Quantitative Comparison of Carbohydrates in Experimentally Induced Connective Tissue of Man, Dog, and Rat
2. Nature of the Carbohydrate Units of Experimentally Induced Connective Tissue of Man, Dog, and Rat
3. Enzymes Involved in Wound Healing: Neuraminidases in Wound Areas of Rats
4. Identification of Specific Glycopeptides and Glycosaminoglycans of Experimentally Induced Connective Tissue of Man, Dog, and Rat

(b) Electron Microscope Studies of the Healing Wound

1. Electron Microscopic Studies on the Effect of Temperature on the Molecular Configuration and Integrity of Carp Swimbladder Acid-soluble Tropocollagen Molecules
2. Electron Microscopic Studies on the Structuring and Growth Aspects of Natural Collagen Fibres and Reconstituting SLS Fibres
3. Electron Microscopic Studies on the Effects of Bases on the Structuring Unit of Collagen Fibres.
4. Electron Microscopic Studies on the Compatibility of Tropocollagen Molecules of Differing Species of Origin in the Fabrication of Collagen Fibres in vitro.
5. New Observations on the Genesis of SLS and Native Collagen Fibres
6. Completed Studies under Editorial Review: A Corpuscular Structural Unit of Solid State Collagen and the Fine Structure of Experimentally Induced Connective Tissue Complexes in the Human

John A. Schilling, B.N. White, M.S. Lockhart, K.M. Richter, C.A. Nau.

Annual Report, Dept.of the Army, Contract No. DA-49-193-MD-2811, 15 July 1973.

John A. Schilling, M. D.

WOUND HEALING

Annual Reports

17. Wound Healing: Biochemical Pathways, Ultrastructure, and Clinical Studies

(a) Biochemical Investigation of the Healing Wound

1. Previous Studies in the Biochemical Section
2. Studies of Insoluble Collagen of Experimentally Induced Connective Tissue of Man, Dog, and Rat
3. Further Studies of the Glycopeptides of the Matrix of Experimentally Induced Connective Tissue of the Rat
4. Comparative Studies of Wound Connective Tissue and Adjacent Host Connective Tissue in the Dog and Rat
5. Exploring the Feasibility of Other Wound Models: The Dacron "Weavenit" Vascular Prosthesis

(b) Electron Microscope Studies of the Healing Wound

1. Electron Microscopic Studies on the Effect of Temperature on the Molecular Configuration and Integrity of Carp Swimbladder Acid-soluble Tropocollagen Molecules
2. Electron Microscopic Studies on the Structuring and Growth Aspects of Natural Collagen Fibres and Reconstituting SLS Fibres
3. Electron Microscopic Studies on the Effects of Bases on the Structuring Unit of Collagen Fibres
4. Electron Microscopic Studies on the Compatibility of Tropocollagen Molecules of Differing Species of Origin in the Fabrication of Collagen Fibres in Vitro.
5. New Observation on the Genesis of SLS and Native Collagen Fibres
6. Final Summation of Electron Microscope Work

John A. Schilling, B.N. White, M.S. Lockhart, Kenneth M. Richter, Carl A. Nau

Annual Report, Dept. of the Army, Contract No. DA-49-193-MD-2811, 15 July 1974.

18. Wound Healing: Biochemical Pathways, Ultrastructure, and Clinical Studies

Biochemical Investigation of the Healing Wound

1. Previous Studies (1953-1973) in the Biochemical Section
2. Recent Studies (1973-1974) of Insoluble Collagen of Experimentally Induced Connective Tissue of the Rat
3. Recent Studies (1973-1974) of the Glycopeptides of the Matrix of Experimentally Induced Connective Tissue of the Rat
4. Recent Comparative Studies (1973-1974) of Wound Connective Tissue and adjacent Host Connective Tissue in the Dog and Rat
5. Recent Studies (1973-1974) of the Feasibility of Other Wound Models: The Dacron "Weavenit" Vascular Prosthesis

WOUND HEALING

Annual Reports

6. Current Studies (1975-1976) of Enzymes Involved in Wound Healing
John A. Schilling, M.D., and Patrick D. Goldsworthy, Ph.D.
Annual Report, Dept. of the Army, Contract No. DAMD 17-75-C-5022-Mod-P-602, July, 1976. (Report covers two-year period from July, 1974 to July, 1976).

19. Wound Healing: Biochemical Pathways, Ultrastructure, and Clinical Studies.
John A. Schilling, M.D., and Patrick D. Goldsworthy, Ph.D.
Annual Summary Report, Dept. of the Army, Contract No. DAMD 17-75-C-5022-Mod-P-602, July, 1978. (Report covers period from July, 1976 to July, 1978).

END

FILMED

9-83

DTIC